**PRODUCTION OF YOGURT FROM SOYMILK AND COW MILK USING PINEAPPLE (*Ananascomosus*) AS SWEETENERS**

**BY**

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**A**

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**CERTIFICATION**

 This is to certify that Eze Gloria Uchenna an undergraduate student in the department of Biotechnology and Applied biology with registration number U14/NAS/BTG/024 under the supervision of Prof. James Ogbonna has successfully completed the research required for the award of Bachelors of Science (B. Sc.) Degree in biotechnology in Godfrey Okoye University.

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The research was undertaken by Eze Gloria Uchenna (U14/NAS/BTG/024) and approved by department of Biotechnology and Applied biology, Godfrey Okoye University Enugu in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc) Degree in Biotechnology.

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**DEDICATION**

This research work is dedicated to God Almighty who in his divine support has made this project a success and to my ever loving sisters.

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**ABSTRACT**

The composite soymilk yogurt flavoured with pineapple as sweetener was produced from composite blend of dano milk and soymilk. It was pasteurized at 850c for 5 minutes. It was cooled , homogenized and inoculated with mixed yogurt starter culture of *Lactobacillus bulgaricus,* and *Lactobacillus acidophilus* at 250c for 24 hours. The proximate, physicochemical properties and sensory evaluation of the composite yogurt were determined. In the proximate, the study reveals that the combination of dano milk and soymilk increased the level of the protein content. This can be seen in the sample 50%S+M with the protein content of 13.23.The fat content of the product equally decreased upon introduction of the sweetener (pineapple). The sensory score of the yogurt samples reveal there was a significant difference (P>0.05) in terms of the taste, texture, and overall acceptability between the sample M100%(control) and the composite soy-yogurt sample (50%M+50%S+P, 100%S,100%S+Pand 100%m+p) except for sample 50%M+50%S that was not significantly difference (P<0.05) from the control. Therefore soymilk can be substituted up to 50% with dano milk yogurt preparation without affecting the physicochemical and sensory properties of the yogurt.

**CHAPTER ONE**

**1.0 INTRODUCTION**

Interest in functional foods has recently increased among consumers due to a greater consciousness of health and nutrition; as well as the need to cure diseases and also the increasing scientific evidence of their effectiveness. Fermented products are a significant part of many indigenous diets.

Yoghurt is a Turkish name for a fermented milk product. It is originated by early normadic herdsman, especially in Asia, Southern and Eastern Europe. Yoghurt is made by adding a culture of acid forming bacteria to milk that is usually homogenized, pasteurized and fermented. Yoghurt is defined as a fermented milk product that evolved empirically some centuries ago by allowing naturally contaminated milk to sour at a warm temperature, in the range of 40-50 °C (Kwon et al., 2011). The micro-organisms which are used conventionally in this process are referred to as “Starter Culture”. They include *Lactobacillus delbrueckii* subsp. Bulgaricus and *Streptococcus thermophilus*. The average size of *Lactobacillus bulgaricus* ranges from 0.8 to 1.0μm in diameter (Rakis .,1978 , Sanfu. , 2009).

During the fermentation, hydrolysis of the milk proteins occurs, the pH drops, the viscosity increases and bacterial metabolites are produced that contribute to the taste and possibly to the health promoting properties of yoghurt. The sugars are fermented by the bacteria into lactic acid, which causes the formation of the characteristic curd. The acid lowers the pH of the yoghurt and restricts the growth of food poisoning bacteria (putrefactive or pathogenic).

Not only is yoghurt a wonderful quick, easy and nutritious snack, but also research evidence point to the fact that milk and yoghurt may actually add years to life as found in some countries where fermented dairy products are a dietary (Wolf.,1978) Several health benefits have been reported for traditional yoghurt and this healthy image is enhanced by supplementation with probiotic bacteria ( ; Magenis, et al 2006).

Soymilk is an aqueous extract of soya beans (*Glycine max*) and is quiet similar in appearance to cow milk (Agure-Dam, 1997). It is commonly characterized as having a beany, grassy or soy flavor, which reportedly can be improved by lactic acid fermentation, as in yoghurt-like products Microorganisms possess endogenous β-glucosidases which can be utilized to hydrolyze predominant isoflavone glucosides in soymilk to improve biological activity.

 It has been reported that probiotic organisms including *Bifidobacteria* and some other lactic acid　bacteria hydrolyze isoflavone glycosides into corresponding aglycones (Cheng., 1998).

Recent reports indicate that some probiotic bacteria could better compete with yoghurt cultures in a soy-based substrate. Soy has been examined as a substrate for the *Lactobacillus* species such as *L. Casei, L. helveticus, L. fermenti, L. fermentum and L. reuteri* (Garro et al., 1999; Murti et al., 1993b;( Chumchuere and Robinson, 1999; Garro et al., 2004; Tzortzis et al., 2004).

 The problems of which is beany flavor can be improved by lactic fermentation, so production of fermented soymilks such as soy yoghurt is important (Nsofor et al., 1992; AbdEl Khair, , 2009).

Due to continuous increase in the population and inadequate supply of animal milk protein leading to malnutrition in Nigeria many research works have been geared finding alternative protein source from legume.

Soymilk can serve as a very good alternative to the expensive cow milk as it contains all the essential amino acid even though some are in a low concentration

It is well known from experiment that diets containing containing casein or other animal protein could induce elevation of plasma total LDL cholesterol concentration but this can be prevented by vegetable protein such as soy protein (caroll and kurowska 1998)

**1.2 HISTORY OF THE YOGURT MAKING**

Milk fermentation is one of the oldest methods practiced by the human beings to preserve milk with an extended shelf life. The exact origination of milk fermentation is not clear; however, it seems that it is dated back to the dawn of the civilization.

It has been reported that the early civilizations such as the Samarians, Babylonians, Pharoes and Indians were well advanced in agricultural and animal husbandry practice. This can be supported by the findings of Copley et al., 2003 in which the dairy fat residues were found in pottery fragments from Neolithic Bronze-age and Iron-age settlements, which suggests that the practice of dairying had existed in Britain approximately 6500 years ago.

However, it is questionable that the milk fermentation was practiced during this period. Therefore, the origin of the fermented milk products including yogurt remains unsolved. It has been reported that the Anatolian goatherds conserve their milk by thickening as they used to dry it in the sun and transport in animal stomachs. It is generally accepted among the historians that the fermentation of milk is discovered accidentally by the Neolithic people of Central Asia when they stored milk in primitive methods such as in sheep-skin bags in warm climates.

 With reference to yogurt, it can be suggested that it has been evolved in Turkey as the term “yoghurt” has been derived from a Turkish verb, “jugurt” that means “to be curdled or coagulated”.(Belleville, 2002)

The earliest writings about yogurt can be found from those of Pliny who lived in the first century A. D. and wrote about ancient barbarous nations that knew how to thicken the milk into a substance with an agreeable acidity. According to the literature,(Douglass et al.,2006) the founder of the Mongol empire, Genghis Khan and his armies lived on yogurt and spreading of this news among the people had made the yogurt consumption to spread throughout the East.

 Moreover, according to the Persian tradition, Abraham owed his fecundity and longevity to the regular ingestion of yogurt, and the emperor Francis I of France was said to be cured of severe diarrhea by consuming yogurt made of goat milk leading to introduce the health benefits of yogurt into the western world in 1542(Howell and Caldwell, 1978)

The first industrialized production of yogurt took place in 1919, in Barcelona, Spain at a company named Danone. Yogurt was firstly introduced to the USA in the early 20th century in the form of tablets especially designed for those with digestive intolerance. However, it became popular in the North America when Danone, a small-scale yogurt factory started manufacture of yogurt in New York in 1940(Fennel, 1966)

Even though, yogurt has been evolved for centuries, it was subjected to a significant and dynamic evolution process in the 20th Century to originate a vast array of products. For instance, fruit yogurts, yogurts with fruit on bottom and blended yogurts were introduced in 1937, 1947 and 1963 respectively. It seems that the evolution process of yogurt has taken place in different regions of the world once it had been originated in the Central Asia. This might be the reason of having different types of yogurts and yogurt-like products in different names.

**1.3 STATEMENT OF THE PROBLEM**

 Animal protein (milk) are more expensive in Nigeria and not easily affordable by all for yogurt production necessitates the need to seek for ways of producing yogurts from plant protein (soy beans).Combination animal protein provides more complete amino acid , hence better nutrition .

Improving product nutrient value by fruit addition and increased new product variety. Reduce postharvest losses of soybean due to underutilization in Nigeria.

Therefore , many researches have been carried out on yogurt but none have being established on using pineapple as sweetener, aimed at achieving the aforementioned solution.

**1.4 AIM/OBJECTIVES**

To produce yogurt from plant protein (soymilk)

To evaluate the sensory attributes of yogurt made from soymilk and cow milk using pineapple as sweetener.

To determine the proximate composition attributes of the yogurt made from soymilk and cow milk using pineapple as sweetener.

To check if there is a significant difference in the texture, appearance, colour, taste and general acceptability of the yogurt produce using ANOVA

To check the proximate contents of the samples produced

**CHAPTER TWO**

 **2.0 LITRETURE REVIEW**

 **2.1 YOGURT**

Yoghurt is an excellent source of calcium and high in protein quality but it contains very little iron which is common among all dairy products. It is produced from fermented milk, which is rich in sugars (lactose) (Tull, 1996). Yoghurt generally contains at least 3.25 % milk fat and 8.25 % solids non fat. Yoghurt can be low fat (0.5 - 2 %) or non fat (less than 0.5 % milk fat) which is more preferred because of health claims.

Yoghurt is one of the best known foods that contain probiotics, which are living micro-organism that when ingested in sufficient amount exerts beneficial effects on the normal microbial population of the gastrointestinal tract (Bourlioux *et al.,* 2003)*.*

The aroma, taste and body of yoghurt and other cultured dairy products can vary depending on the type of milk, culture, amount of milk fat and non fat milk solids, fermentation process and temperature used.Yoghurt could be stored safely longer than milk. Therefore, the production of yoghurt is a way of preserving fresh milk. The fermentation of the lactic acid in milk occurs as a result of the action of the lactic acid bacteria, *Lactobacillus* *bulgaricus* and *Streptococcus thermophilus*. These strains secrete an enzyme called galactosidase that allows them to pre-digest part of the lactose (the sugar in milk). This is why generally people with lactose intolerance can easily digest yoghurt.

Yoghurt is traditionally prepared by fortifying whole or skimmed milk by evaporation or heating to 85 - 95 0C for 10 - 30 minutes and inoculating with lactic acid bacteria and incubating at 42 - 45 0C. (Routray and Mishra, 2011). Many consumers associate yoghurt with good health. The popularity of yoghurt as a food component has been linked to its sensory characteristics (Routray and Mishra, 2011). Along these, the flavour of yoghurt has played an important role in increasing its consumers demand.

**2.2 TYPES OF YOGURT**

Yogurts can be divided into a number of types based on how they are made. These types of yoghurt include:

**Balkan-style yogurt**

Balkan-style yogurt is also known as set-style yogurt which has a characteristic thick texture and made in small and individual batches after poured the warm cultured mix into containers following by incubation without any stirring for over 12 hours or more until the desired thickness and creaminess is attained. This type of yogurt is used in making Balkan meat-based recipes, as a substitute for sour cream, as salad dressing or topping for Mediterranean dishes such as moussaka, spanakopita, pita sandwiches with slices of meat or chicken. In addition, it can also be consumed as a regular yogurt, sweetened with chopped fruits, sugar or honey or served with granola for breakfast.

**Greek-style yogurt**

This is also known as Mediterranean-style yogurt made of either from partially condensed milk or by staining whey from plain yogurt to make it thicker and creamier. Due to its thick texture, it tends to hold up better upon heat than regular yogurt and thus utilizes as a main ingredient in making thick dips such as tzatziki. Although it is a delicious product, it claimed to have a high amount of fat including high content of saturated fat. However, it is likely to be a rich source of vitamin A. 150g serving will provide one fifth of the Recommended Daily Allowance of vitamin A . Greek-style yogurts are available, in full fat and low fat versions, many cooks and bakers used to make their own strained yogurts with the intension to utilize in their cuisines in fresh form.

**European –style yogurt**

European-style yogurt is a type of stirred yogurt with a characteristic creamy and smooth texture and is made by incubating the yogurt mixture in a large vat instead of individual cups, followed by cooling and then stirring in order to obtain a creamy texture most often with added fruits (blueberries, strawberries, mango, and peach) and flavors. Yogurts of this style are slightly thinner than that of the Balkan-style and set yoghurt and can be incorporated into cold beverages and

 **French-style yogurt**

This style of yogurt is also known as custard-style yogurt made by direct culturing in the pot according to a French culture and process which characterized with a pudding-like texture. Sometimes French-style yogurts are flavored with fruit pieces which are stirred into the mixture, most commonly with strawberries and blueberries or a mixture of both. It is said to be an ideal source of iron, protein and vitamin A.

**Fruit yogurt**

There are two kinds of fruit yogurts: one has the fruits set at the bottom of the packaging (sundae-style yogurt) while the other has the fruit uniformly distributed within the yogurt itself (Swiss-style yogurt). Fruit pieces or pulp are added at production stage that produces variety of tastes while increasing consumer appeal and sweetness.

**2.3 BACKGROUND OF SOYBEAN PLANT**

The cultivation of soybeans originated in Eastern Asia and soybeans have long been used as a food before the existence of written records. The first Chinese record describing soybeans is in the Chinese book. *Pen Ts'ao Kong* *Mu, was* written in 2838 B.C. (Smith and Circle, 1978a). The soybean *Glycine max* (L.) *Merr* is a member of the family Leguminosae, subfamily Papilionoideae. The soybean plant has alternate, tri-foliolate leaves except at the first nodes.

The leaves, stems, and pods are normally covered with a gray or brown (tawny) pubescence, which is very noticeable at maturity and is useful in variety identification. Seeds of most current soybean varieties are nearly spherical. Average seed weight is 120 to 180 mg of which the seed coat contributes about 10%(Howell and Caldwell, 1978). The soybean is Asiatic in origin but has been adapted to latitudes from 0 to more than 50 degrees. The United States now produces about 60% of the world acreage which accounts for about 75% of world production. Production in the United States is almost entirely in the North Central States, the lower Mississippi Valley, and the South Atlantic States (Howell and Caldwell, 1978). The first mention of soybeans as a crop in American literature was in 1804 by James Mease in Willies Domestic Encyclopedia, first American Edition, in which he suggested that soybeans could be produced in Pennsylvania (Smith and Circle, 1978a). The Perry Expedition in 1854 brought back varieties of soybeans from Asia which was distributed to interested people. Some European countries, especially England, started importing soybeans from Manchuria in 1908 to supplement short supplies of cottonseed and flaxseed. The beans were processed into oil and meal. The oil was used mostly for the manufacture of soap and the cake or meal for feeding dairy cattle. The success in the utilization of soybean cake and oil in Europe was an inspiration for similar usage in the United States (Smith and Circle,1978a). In the early 1900's, U.S. interest in soybean processing developed. In 1911, Hernan Meyer opened a snail crushing plant in Seattle, Washington, processing beans imported from Manchuria. Initially soybeans, including the whole vine, were used as hay and silage. Several years elapsed before the value of the oil and protein as food and feed source was clearly recognized. Commercial processing of the seed was not established until 1922 (Smith and Circle, 1978a). The shortage of industrial and food grade oils and of protein for animal feeds during World War further encouraged the processing of domestic soybeans. During this same period the cultivation of soybeans spread from the Carolinas to Illinois and surrounding states (Smith and Circle, 1978a).

In Nigeria Soybean was first introduced to Ibadan, Oyo State, in 1908 with little or no success in

The rainforest ecology of the State(Fennel, 1966).

In 1928, it was introduced to the savanna area of Northern Nigeria where the soil and climatic conditions supported its production. The crop was successfully cultivated in 1937 for multiplication and commercial production in Benue State (Nyiakura, 1982). Since then, many small-scale farmers in the south central part of the country have continuously incorporated propagation into their cropping systems.

 Benue, Kaduna, Plateau and Niger are the major Soya producing states in Nigeria.

Other Soya producing areas include Nasarawa, Kebbi, Kwara, Oyo, Jigawa, Borno,

Bauchi, Sokoto, Taraba and the FCT. Benue State produced about 44 percent of the national

output while Kaduna State produced 26.7 per cent. The producing States for soybean accounted

for 96 percent of soybean produced in Nigeria.. The high production levels in benue state is both as a result of the large individual farm holdings and the number of producers. However, it was

observed that, in Benue State, soybean is produced as a major crop in every LGA, accounting for

over half of the farm size of individual farmers. While in Kaduna state the production of soybean

is localized in only few LGAs, soybean is produced in all the LGAs in benue State. The LGAs

producing higher quantities of soybean in Ondo and Oyo States are Akure North and Saki West

**2.4 CHEMICAL COMPOSITION OF SOYBEAN SEED**

**CARBOHYDRATES**

The principal sugars of the soybean are the disaccharide sucrose (5.0%), the trisaccharide raffinose (I.1%), and the tetrasaccharide stachyose (3.8%), which has 15% hemicellulose, 4% cellulose and 5.1%other carbohydrates (such as glucose, arabinose, and verbascose) which account for the total carbohydrate content of 34%of whole bean (Kawamura, 1967). Raffinose and stachyose are non reducing sugars without food value unless they have been hydrolyzed by strong acids into their components of galactose, glucose, and fructose. The enzymes which hydrolyze raffinose and stachyose are invertase and emulsion. Invertase hydrolyzes the sucrose portion of the molecule to give melibiose and D-fructose. Emulsin, which contains an a-D-galactosidase, as well as, a p-glucosidase, hydrolyze the melibiose residue to yield galactose and sucrose. Bottom yeasts, which contain both enzymes, can completely hydrolyze raffinose (Smith and Circle, 1978b). Therefore, these indigestible carbohydrates can be converted to digestible mono- or disaccharides with microbial action. Glucose or other reducing sugars are present in green or immature beans in substantial amounts, but they disappear as the beans approach maturity. Starch is seldom found in mature beans but frequently reported in immature beans (Smith and Circle, 1978b). The most fiber, including hemicellulose and cellose, was present in soybean hull.

**PROTEINS**

Protein storage particles, called protein bodies or aleurone grains, vary from 2 to 20 µm in diameter, although many falls into the narrower range of about 5 to 8 µm. Atleast 60-70% of the total protein in the soybean is stored in the protein bodies (Wolf, 1978). Minimum solubility of these proteins occurs at pH 4.2, corresponding to the apparent isoelectrical point of the major proteins. Adjustment of aqueous or dilute sodium hydroxide extracts of defatted meal to pH 4.0-4.2 precipitates about 90% of the extracted protein (Wolf,1978).

The globulins, which include conglycinin (7S) and glycinin (11S), account for approximately 80% of the proteins. The 7S is a quaternary trimeric glycoprotein composed of 3 subunits associated via hydrophobic interactions. The 7S possesses approximately 2 intramolecular disulfide bonds and contains 5% carbohydrate. Thus, it may possess amphiphilic properties for good surface activity and flavor binding. Glycinin (IIS) consists of acidic and basic subunits associated by 2 pairs of disulfide linkage and 6 hydrophobic interactions. The isoelectric point of glycinin is around pH 6.0 at which point glycinin has limited solubility. The IIS also contains 2 free thiol groups, which apparently are located on the surface and can engage in thiol-disulfide interchange (Kinsella, 1985).

The other proteins present in soybean are trypsin inhibitor, hemagglutinin, and lipoxygenases. The trypsin inhibitor possesses a single polypeptide chain and is compact, low in asymmetry and rigid in structure. Hemagglutinin is a glycoprotein. The absence of cystine crosslinks in this glycoprotein suggests that the molecule may be fairly flexible and subject to conformational changes very easily. The enzyme, lipoxygenase, composed from 2 subunits with 58,000 molecular weight.

**LIPIDS**

Lipid deposits, or spherosomes, are interspersed between the protein bodies and are about 0.2-0.5 in diameter (Wolf, 1978). The primary fatty acids present in soybean oil are unsaturated fatty acids, such as oleic acid (22.8%), linoleic and linolenic acids (57.6%), and 14% of the palmitic and stearic acids, while the rest are 0.3% saturated fatty acids with a carbon number less than 14

(Brisson,1981). These fatty acids and glycerol consist of 16.5% lipid in mature dry soybean (Smith and Circle,1978b).

 **NUTRITIONAL ASPECT**

Soybean milk is an ideal hypoallergenic foodstuff for infants who are allergic to human milk and cow's milk. Soybean oil and soy sauce are devoid of allergenic agents (Rackis, 1978). Soybean protein contains various amino acids. The soymilk is lower in essential amino acids of methionine, lysine, leucine, isoleucine, and valine than cow’s milk. Soybean contains several proteins having anti-nutritional properties such as trypsin inhibitors and lectins. Trypsin inhibitors block the pancreatic enzyme, trypsin, which is necessary for proteolytic digestion and protein utilization. Lectins can form complexes with carbohydrates then agglomerate and human cannot utilize these carbohydrates. The nutritional value of soy protein is limited by these molecules, and by the protein's inherent quaternary and tertiary structure.

During the processing of soybeans into food and feed products, these anti-nutritional proteins are greatly reduced or eliminated through denaturation process (Bradford and Orthoefer,1983).

Reduction of disulfide bonds enhances digestibility approximately 2-fold (Kinsella, 1985). Denaturation of soybean proteins by moist heat is well known and has long been used to eliminate anti-nutritional factors in soybean meals and flours used in feeds and foods (Wolf, 1978). Heat processing also enhances palatability and acceptance in soybased foods. Benefits derived from heat processing include, destruction of various anti-nutritional factors (e.g., trypsin inhibitors, hemeagglutinins, goitrogenic factor) and in part, from modification of protein, permitting more complete digestibility and utilization of the growth-limiting sulfur-containing amino acids (Angeles and Marth, 1971). Heating soymilk reduces or eliminates the microbial flora and resultant competition, and thus insures optimal development of the starter organisms and uniformity in the final product (Angeles and Marth, 1971).

Legumes are frequently responsible for producing flatulence in humans. Flatus is composed of gas, which results from fermentation of carbohydrates, primarily undigested stachyose and raffinose. Bacteria present in the human intestinal tract can produce a-galactosidase which hydrolyzes the oligosaccharides (Rackis et al., 1970; Mital and Steinkraus, 1975). Two gases, H2 and CO2, account for most of the flatus. In some humans, methane is produced in high amounts at the expense of H2(Rackis et al., 1970). Additionally the gases produced are transported across the intestinal wall and interfere with carbonic anhydrase which is postulated as further contributing to flatulence (Rackis, 1978).

 **Figure 3.1 Table 1.**

**Comparison of amino acids in the protein of soybean and cow’s milk(g per 100grams of protein)**

|  |  |  |
| --- | --- | --- |
| **Amino Acids**  | **Soybeans**  | **cow’s milk** |
| **ESSENTIAL**  |  |  |
| Tryptophan  | 1.4  | 1.4 |
|  Lysine  | 6.4  | 7.8 |
|  Threonine | 4.0  | 4.6 |
|  Leucine  | 7.7  | 9.9 |
|  Isoleucin  | 5.3  | 6.2 |
| Valine  | 5.3  | 7.0 |
| Phenylalanine  | 5.0  | 5.1 |
| **NONESSENTIAL** |  |  |
| Tyrosine  | 3.7  | 5.6 |
| Cystine  | 1.9  | 0.8 |
|  Glycine  | 4.5  | 1.9 |
|  Alanine  | 5.0  | 3.7 |
| Serine  | 5.8  | 5.8 |
|  Proline  | 5.3  | 9.8 |
| Argentin  | 7.4  | 3.7 |
| Histidine  | 2.6  | 2.7 |
| Aspartic acid  | 12.3  | 8.2 |
| Glutamic acid  | 19.0  | 22.2 |

Deatherage, 1975)

**OTHER SOYBEAN COMPONENTS**

Soybean contains other compounds, among them isoflavones, oxalate, phytic acid and bioactive peptides, which act in different ways in the human body. Isoflavones are a subclass of a large group called flavonoids. Because of its estrogenic activity, also known as phytoestrogens (Cederroth and Nef, 2009). In soybean there are basically three types of isoflavones that are normally present in four different isoforms: glucosides (daidzin, genistin and glycitin); acetylglucosides (acetyldaidzin acetylgenistin and acetylglycitin); malonylglucosides (malonyldadzin, malonylgenistin and malonylglycitin) and structure unconjugated aglycone (daidzein, genistein and glycitein) (Kao et al., 2007; Pereira and Oliveira, 2002).

Soybean is the main source of isoflavones (genistein, daidzein and glycitein), and its concentration range from 1.45 to 4.59 mg/g. Traditional fermented products (miso, tempeh) contain high concentrations of aglycone forms, genistein and daidzein, resulting from the enzymatic action of glucosidase, while non-fermented products such as dried beans, aqueous extracts of soybeans, soy flour and tofu, contain high concentrations of the glucosides, genistin and daidzin (Wang and Murphy, 1994).

The majority of species in the Leguminosae family contain oxalate in one or more of their tissues. The greater concentrations of oxalate in soybean are found in the mature seeds (Ilarsla et al. 2001;Nakata, 2003). The oxalate content of some plants is known to vary by genotype of soybean and possibly within cultivars under different growth conditions. Some Brazilian soybean cultivars, such as UFV-116 and OCEPAR-19 present oxalate content of 0.38 and 0.20 mg/100g, respectively (Esteves et al., 2010). Absorbed oxalate cannot be metabolized by humans and is excreted in the urine. In humans, high urinary oxalate increases the risk of calcium oxalate (CaOx) kidney stones because CaOx is poorly soluble in the urine. Saturated oxalate in solution binds to Ca to form crystals that aggregate, often becoming large enough to block the urinary stream (Massey et al., 1993).

The phytic acid, also known as phytate, inositol hexaphosphate (IP6), myo-inositol, myo-inositol and inositolphosfate, is the main form of phosphorus stocks in soybean which is essential for germination of the grain (Domínguez et al., 2002). During processing, storage, fermentation, germination and digestion of grains and seeds, IP6 may be partly dephosphorylated to produce compounds as pentaphosphate (IP5),tetraphosfato (IP4), triphosphate (IP3) and possibly inositol diphosphate (IP2) and monophosphate (IP1), by action of endogenous phytases (Chiplonkar & Agate, 2005;Domínguez et al., 2002) . Only IP6 and IP5 exert a negative effect on the bioavailability of minerals. The other compounds formed such as IP4 and IP3 has low capacity to bind to the minerals or complexes formed are more soluble. Furthermore, these compounds may have antioxidant properties (Andrade et al., 2010; Domínguez et al., 2002; Martino et al., 2007). During gastrointestinal digestion or food processing of proteins, small bioactive peptides can be released. Food-derived peptides commonly contain 2 to 9 amino acids (Kitts &Weiler, 2003). However, this range may be extended to 20 or more amino acid units (Korhonen & Pihlanto,2003). Numerous biologically active peptides have been identified in foods, and may act as regulatory compounds with hormone-like activities. Soybean is a potential source of bioactive peptides which play an important role in physiological activities, particularly those related to the prevention of chronic diseases. Peptides with other biological activities, such as opioid agonistic and antagonistic, anti--oxidative, anticancer, and immunomodulatory actions have also been identified in soybean (Wang and De Mejia, 2005).

**2.5 FUNTIONAL PROPERTIES OF SOYBEAN**

A food is defined as functional if, “in addition to providing nutrients, it contains bioactive compounds that act promoting health and/or reducing the risk of chronic diseases” (Diplock et al., 1999). In this context, soybean is considered a functional food with high potential. Experimental and epidemiological studies have provided evidence for a variety of health benefits derived from the consumption of soybean and soy food products, This health benefits includes:

**IMPROVEMENT IN SERUM LIPID PROFILE**

The consumption of soybean or its bioactive compounds has been reported to contribute significantly to reducing cholesterol and triglyceride levels in laboratory animals and humans (Esteves et al., 2011; Reynolds et al., 2006; Sirtori et al., 2007; Sirtori et al., 2009). The Soybean and Health beneficial effect on lipemia has been explained by the action of various constituents present in soybean which act via different mechanisms:

*Amino acid protein profile.* The high content of arginine and low methionine content (Wilson et al., 2007) can promote higher levels of nitric oxide and lower levels of homocysteine (Torres et al., 2006), favoring vessel relaxation and reduction of the risk of cardiovascular disease. (Gornik & Creager, 2004). There are suggestions that soy protein exerts hypolipidemic and anti-atherogenic effects because the relationship between amino acids lysine/arginine alters the relationship insulin/glucagon which, when elevated, increases the risk of cardiovascular disease (Demonty et al., 2002; Torres et al., 2006).

*Action of non-digestible peptides*. The presence of peptides from soybean not digested in the gastrointestinal tract, has also explained their hypocholesterolemic effect. Peptides increase the fecal excretion of steroids, elevating both the hepatic synthesis of bile acids and receptors of LDL-c, as well as the uptake and oxidation of cholesterol in the liver (Belleville, 2002). Hypocholesterolemic effect has been found in peptide Leu-Pro-Tyr-Pro-Arg, a protein fragment derived from soybean glycinin, which reduced serum cholesterol in mice (–25.4%in total cholesterol and –30.6% in LDL-c) (Yoshikawa et al., 2000). This peptide has structural homology to enterostatin (Val-Pro-Asp-Pro-Arg). Although both have hypocholesterolemic activities, enterostatin did not increase excretion of bile acids in feces, suggesting that they may act by different mechanisms (Takenaka et al., 2004).

*Bioactivity of isoflavones.* Isoflavones, acting on the **β**-estrogen receptors present in the liver, lead to increased number of hepatic receptors of LDL-c, and favors the catabolism of cholesterol and **β**-oxidation of fatty acids (Dewell et al., 2002; Douglas et al., 2006; Torrezan et al., 2008). The antioxidant effect of isoflavones can act protecting the oxidation of copper dependent LDL-c and favor a serum lipid profile associated with protection against atherosclerosis (Teixeira Damasceno et al., 2007).

*Hypolipidemic action of storage proteins.***β**-conglycinin and glycinin showed hypolipidemic effect, by increasing the fecal excretion of fatty acid, induction of **β**-oxidation in the liver and decreased hepatic synthesis of fatty acids by down regulation of fatty acid synthesis and up regulation of liver VLDL receptors (Duranti et al., 2004; Fukui et al., 2004; Moriyama etal., 2004).

*Hypolipidemic action of phytate.* The phytate intake improves the serum lipid profile and reduces the hepatic lipid deposition in animal model of atherosclerosis (Lee et al., 2007). It is known that phytate exerts effect on hepatic glucose-regulating enzyme activities and reduce the risk of high fat diet-induced hyperglycemia (S.M. Kim et al., 2010).

The evidences commented above show that the beneficial effects of soybean on lipid profile improvement is mediated by various constituents present in the grain and thus the intake of whole soybean has potential to exert greater effect in comparison with supplementation of their components. This idea is supported by some evidence. The hypocholesterolemic effect of soy consumption may be attributed not only to the presence of bioactive compounds (intrinsic effect) but also to replacing animal foods rich in saturated fat and cholesterol (extrinsic effect). A recent study estimated the intrinsic and extrinsic effects of soybean to reduce cholesterol and verified that the combined effects are important to reduce cholesterol in approximately 4% (Jenkins et al., 2010). In fact, the synergistic action of amino acids and isoflavones in improving lipid metabolism and activation of the LDL-c has been demonstrated (Bertipaglia Santana et al., 2008).

**PROTECTION AGAINST OXIDATIVE STRESS**

Isoflavones and its metabolites formed by bacterial action in the intestinal lumen, have structural determinants to exert strong antioxidant activity (Arora et al., 1998). Studies in humans have shown that the antioxidant effect of soy products. The intake for 2 weeks, of soy bars containing

7 and 12 milligrams of daidzein and genistein, respectively, increased the resistance of oxidation of isolated LDL (Tikkanen et al., 1998). Although the ingestion of a product isolated soy containing protein and isoflavones did not show antioxidant effects of plasma and LDL-c (Steinberg et al., 2003). The antioxidant effects are mediated by direct elimination of free oxygen radicals by isoflavones (Teixeira Damasceno et al., 2007) and by increasing the concentrations of antioxidant enzymes, improving the body's antioxidant defense (Engelman et al., 2005; Rios et al., 2008). It is believed that a diet rich in isoflavones causes LDL-c to become resistant to peroxidation, that is responsible for the generation of a cascade of events that produces atherosclerotic plaques, making the consumption of soy a protective factor against atherosclerosis (Mateos-Aparicio et al., 2008; Sacks et al., 2006 Lee et al., 2007; Martinez Dominguez et al., 2002 ).

It is known that the protective effect of soybean against oxidative stress involves the action of several bioactives compounds and once again, it is reasonable to state that soy intake may offer greater antioxidant protection mediated by the synergistic effect of several components: isoflavones and their metabolites generated in the intestinal lumen, peptides and phytic acid. In addition, the action of other antioxidants such as vitamin E and minerals like copper, iron, zinc and manganese, although not present in high concentrations in soybean, help in the antioxidant protection of the body. All these factors suggest that eating whole soybean would be more effective than the intake of their isolated components (Quirrenbach et al., 2009).

**EFFECT ON GLYCEMIC CONTROL**

Soybean has shown a potential to exert physiological effects through mechanisms that stimulate insulin production and decrease the glycemic index of diet, suggesting the possibility of their health claim to act in the prevention and control of diabetes and obesity and its metabolic complications. The effect of isoflavones in reducing blood glucose may be explained by the stimulus by genistein to pancreatic cells which increases insulin production and consequently also the glucose uptake by cells (Esteves et al., 2011). These effect has been demonstrated in studies with animals that received genistein (Jonas et al., 1995) and also in an *ivitro* study, which used adipocytes and insulinoma cells, showing that bioactive compounds formed from isoflavonoids and soy peptides during the fermentation process to produce meju, activated signaling cascades that stimulated insulin release (Kwon et al., 2011).

The dietary fiber contained in soybeans is effective in controlling blood glucose (Penha et al., 2007). It can reduce the rate of emptying of the digestive tract, increase the rate of peristalsis of the bowel and slow down the rate of glucose uptake and therefore its importance in the control of blood glucose (Takahashi et al., 2003). In a clinical study, the intake of soybean fiber resulted in decrease in the blood glucose levels after ingestion of a glucose solution. Thus, soy fibers may adsorb glucose in the intestine and slow their release for absorption (Messina et al., 1992).

 A cake made with whole soybean flour showed low glucose and insulin indexes in study with a group of 20 individuals (Oku et al., 2009). Phytate also shows potential in controlling diabetes, as demonstrated by its hypoglycemic effect in diabetic mice (S.M. Kim et al., 2010; Lee et al., 2007). Lee (2006) observed that consumption of diets containing phytate reduced the high blood glucose levels and glycated hemoglobin in diabetic mice.

In summary, various bioactive soy compounds may act on mechanisms that improve the metabolism of carbohydrates, working in reducing the risk of diabetes and metabolic complications of obesity. As an adjunct to control diabetes, it stands out the importance of dietary fiber to improve the glycemic index of diet as well as the antioxidant components to minimize the oxidative stress that is present in this disease. However, in diabetic individuals, studies are needed to prove whether pancreatic cells of these individuals are responsive to stimulation of soy isoflavones to increase insulin release.

**CHEMOPREVENTIVE EFFECTS**

Epidemiologic studies have demonstrated the direct association between the protection of breast cancer and soy intake (Dong and Qin, 2011). This effect has been attributed to the bioactivity of isoflavones and their estrogenic effect. However, intervention studies do not always demonstrate benefits of supplementation of isolated soy isoflavones in reducing the Nutritional and Bioactive Compounds of Soybean: Benefits on Human Health risk of breast cancer. In a study with 406 premenopausal women, who received 80 or 120 mg/day of isoflavones or placebo, during two years, did not showed modification in mammographic density, which is a marker of risk of breast cancer. However, there was a significant reduction of the marker over time (Maskarinec et al., 2009). There is evidence that early exposure to the bioactive compounds of soybean, via maternal diet, may alter the paracrine signaling in adipocytes of breast tissue and increase the differentiation of epithelial tissue, with implications in prevention of breast cancer associated with obesity (Su et. al., 2009). In prostate cancer, the chemopreventive effect of the soybean mediated by mechanisms of modulations of gene expression was evidenced in a study in which isoflavones derived from soybean cake exhibited antiproliferative effects on prostate cancer cells, decreasing the expression of cyclin B1 (Wang et al., 2009). A recent study investigated the effect of administration of soy isoflavones as an adjunct in hormone therapy and verified that for postmenopausal women there was lower risk of cancer recurrence, for a monitoring period of 5.1 years (Kang et al., 2010).

Although components of soybean have demonstrated potential to act as antiproliferative agents, their effect in reducing cancer risk is still controversial. It is possible that the metabolic imprint is the main mechanism to explain the inverse association between cancer and soy intake. In fact, it was found that for breast cancer, the association is present for the Asian population and not for the western population (Dong and Qin, 2011). For this reason, the habit of soy intake by the population through successive generations could be a useful strategy to obtain the chemopreventive benefits of the bioactive compounds in soybeans.

**EFFECTS ON OBESITY AND METABOLIC SYNDROME**

Several anorectic peptides have been already identified to exert anti-obesity activity throughdecreasing food intake, fat and lean body mass, and body weight (Challis et al., 2004). Forexample, Leu-Pro-Tyr-Pro-Arg, a peptide from soybean glycinin A5A4B3 subunit (Takenakaet al., 2004) and Pro-Gly-Pro have been found to have anorectic activities.The consumption of soy protein may also lead to low hepatic deposits of triglycerides(Ascencio et al., 2004), reducing the risk of hepatic lipotoxicity and therefore of steatosis (Tovar et al., 2005). In animals, soybean protein modulated the expression of genes that regulated lipid metabolism and thyroid hormone, promoting weight loss (Simmen et al., 2010). These mechanisms of action may attenuate metabolic changes that occur in obesity, which are related to insulin resistance (Oh et al., 2011).

Soy protein isolate was found to lower plasma triglycerides, increase adiponectin (Nagasawa et al., 2003), accelerate lipid metabolism, and decrease body fat in obese rats and mice (Aoyama et al., 2000). Thus, there is enough scientific evidence to motivate soybean research for exploring their potential for its use as a co adjuvant in the treatment of obesity and its complications.

**OTHER DIVERSE EFFECTS**

Soybean peptides with immunomodulatory activities have also been identified from soybean protein hydrolysates. For example, immunostimulating peptide preventing the alopecia induced by cancer chemotherapy has been isolated from an enzymatic digest of soybean protein (Tsuruki et al., 2005; Tsuruki and Yoshikawa, 2004). Recently, it was shown that a lectin of 48-kDa purified soybean black Koreana inhibited the activity of reverse transcriptase of HIV-I with an IC (50) of 1.38 micromolar (Fei Fang et al., 2010). Although there are suggestions of the benefits of soy on bone health, clinical studies do not always prove such effects (Alekel et al., 2010; Kenny et al., 2009). A prospective cohort study of the Chinese population showed that the intake of soybean and its products was associated with protection against hip fracture in women but no association in men (Koh et al., 2009). There are doubts if the intake of soybean can provide superior beneficial effect on bone health (Reinwald and Weaver, 2010).

Prebiotic effect with improvement in the balance of minerals especially calcium and magnesium was demonstrated in rats, which consumed the 120mg/day/animal of galactooligossacarides contained in soybean serum obtained from by-product of the manufacturing of tofu (Tenorio et al., 2010). Soy isoflavones derived from soybean cake exhibited effect in inhibiting apoptosis and inflammation induced by UVB in *in vitro* and *in vivo* mice models, suggesting bioactivity of soy components against aging (Chiu et al., 2009). In animal models of neurotoxicity induced by beta-amyloid, neuroprotective effect of soyisoflavones were demonstrated, indicating the possible benefit of bioactive compounds as co adjuvant in disease (Ma et al., 2009). Study on animals showed that the soybean extract rich in isoflavones increased the epithelium and vaginal collagen (Carbonel et al., 2011).

**2.5.1 PINEAPPLE (*Ananas comosus)***.

Pineapple (*Ananas comosus*, Bromeliaceae) is a wonderful tropical fruit having exceptional

Juiciness, vibrant tropical flavour and immense health benefits. Pineapple contains considerable

calcium, potassium, fibre, and vitamin C. It is low in fat and cholesterol. It is a source of vitamin C which is the body's primary water soluble antioxidant, against free radicals that attack and damage normal cells (Belleville 2002)

It is also a good source of vitamin B1, vitamin B6, copper and dietary fiber. Pineapple is a digestive and natural Anti-Inflammatory fruit. A group of sulfur-containing proteolytic (protein digesting) enzymes (bromelain) in pineapple aid digestion.(Marcela, 2012). Fresh pineapples are rich in bromelain used for tenderizing meat. Bromelain has demonstrated. Significant anti- inflammatory effects, reducing swelling in inflammatory conditions such as acute sinusitis, sore throat, arthritis and gout and speeding recovery from injuries and surgery. Pineapple enzymes have been used with success to treat rheumatoid arthritis and to speed tissue repair as a result of injuries, diabetic ulcers and general surgery. Pineapple reduces blood clotting and helps remove plaque from arterial walls. Studies suggest that pineapple enzymes may improve circulation in those with narrowed arteries, such as angina sufferers. Pineapples are used to help cure bronquitis and throat infections.

 It is efficient in the treatment of arterioscleroses and anemia. Pineapple is an excellent toner; it combats loss of memory, sadness and melancholy. Pineapple fruits are primarily used in three segments, namely, fresh fruit, canning and juice concentrate with characteristic requirements of size, shape, colour, aroma and flavour.(saito et al ,2003 )

**Potential Anti-Inflammatory and Digestive Benefits**

Bromelain is a complex mixture of substances that can be extracted from the stem and core fruit of the pineapple. Among dozens of components known to exist in this crude extract, the best studied components are a group of protein-digesting enzymes (called cysteine proteinases). Originally, researchers believed that these enzymes provided the key health benefits found in bromelain, a popular dietary supplement containing these pineapple extracts. In addition, researchers believed that these benefits were primarily limited to help with digestion in the intestinal tract. However, further studies have shown that bromelain has a wide variety of health benefits, and that many of these benefits may not be related to the different enzymes found in this extract. Excessive inflammation, excessive coagulation of the blood, and certain types of tumor growth may all be reduced by therapeutic doses of bromelain when taken as a dietary supplement. Bromelain extracts can be obtained from both the fruit core and stems of pineapple.

Potentially important chemical differences appear to exist between extracts obtained from the stem versus the fruit core. However, the practical relevance of these differences is not presently understood. Most of the laboratory research on bromelain has been conducted using stem-based extracts, however. Although healthcare practitioners have reported improved digestion in their patients with an increase in pineapple as their "fruit of choice" within a meal plan, there are no published studies that document specific changes in digestion following consumption of the fruit versus supplementation with the purified extract. However, it is suspected that the fruit core will eventually turn out to show some unique health-supportive properties, including possible digestion-related and anti-inflammatory benefits.(Kitts and weiler 2003)

**Antioxidant Protection and Immune Support**

Vitamin C is the body's primary water-soluble antioxidant, defending all aqueous areas of the body against free radicals that attack and damage normal cells. Free radicals have been shown to promote the artery plaque build-up of atherosclerosis and diabetic heart disease, cause the airway spasm that leads to asthma attacks, damage the cells of the colon so they become colon cancer cells, and contribute to the joint pain and disability seen in osteoarthritis and rheumatoid arthritis.

This would explain why diets rich in vitamin C have been shown to be useful for preventing or

reducing the severity of all of these conditions. In addition, vitamin C is vital for the proper function of the immune system, making it a nutrient to turn to for the prevention of recurrent ear infections, colds, and flu.

**Manganese and Thiamin for Energy Production and Antioxidant Defenses**

Pineapple is an excellent source of the trace mineral manganese, which is an essential cofactor in a number of enzymes important in energy production and antioxidant defenses. For example, the key oxidative enzyme superoxide dismutase, which disarms free radicals produced within the

mitochondria (the energy production factories within our cells), requires manganese. Just one cup of fresh pineapple supplies 128.0% of the DV for this very important trace mineral. In addition to manganese, pineapple is a good source of thiamin (Vitamin B1) that acts as a cofactor in enzymatic reactions central to energy production.

**Protection against Macular Degeneration**

Fruits are more important than carrots for eye sight. Data reported in a study published in the

*Archives of Ophthalmology* indicates that eating 3 or more servings of fruit per day may lower the risk of age-related macular degeneration (ARMD), the primary cause of vision loss in older adults, by 36%, compared to persons who consume less than 1.5 servings of fruit daily. (Marcela,2012)In this study, which involved over 110,000 women and men, researchers evaluated the effect of study participants' consumption of fruits; vegetables; the antioxidant vitamins A, C, and E; and carotenoids on the development of early ARMD or neovascular ARMD, a more severe form of the illness associated with vision loss. While, surprisingly, intakes of vegetables, antioxidant vitamins and carotenoids were not strongly related to incidence of either form of ARMD, fruit intake was definitely protective against the severe form of this vision-destroying disease. Three servings of fruit may sound like a lot to eat each day, but pineapple can help you reach this goal. One of the juiciest fruits that is absolutely a delight to eat is the pineapple. It can be taken with whipped cream, custard or just like that. Pineapple juice is equally yummy and refreshing and is one of the favorite drinks of many people during hot weather. The best part about pineapples is that it is loaded with nutrients and beneficial enzymes, which ensures that you not only have a healthy body but also a glowing complexion. Pineapple is known to be very effective in curing constipation and irregular bowel movement. This is because it is rich in fibre, which makes bowel movements regular and easy. It works effectively in getting rid of nausea and vomiting sensation. It has virtually no fat and cholesterol and is loaded with essential nutrients and vitamins that are needed by the body for overall growth and development (Jerkins et al 2010)

Juice from fresh pineapple can be used to relieve bronchitis, diphtheria and chest congestion.

Pineapple is effective in getting rid of intestinal worms and also keeps the intestines and kidneys clean. It is effective in flushing out the toxins from the body, thus making the metabolism healthy.

**2.5.2 LACTIC ACID BACTERIA**

It is a widespread microorganism and easily isolated from carbohydrates rich foods. *Lactobacillus* is the most common bacterium which is used for the human welfare. These bacteria help in the digestion of food, and produce active compounds like vitamin K and bacteriocins, and maintain the balances of normal intestinal flora. It is more frequently used for protective culture in foods to inhibit the unwanted microbial flora (Aureli *et al*., 2003). The LAB is grouped in *Clostridium* branch of Gram-positive bacteria. It is micro-aerophilic, non-spore forming and catalase negative bacteria. It is divided in cocci and rod shapes based on its

morphology, such as *Bacillus, Lactococcus Pediococcus* and *Enterococcus*. It produces lactic acid from glucose.

The G+C content is usually between 32 and 51 mol %. Lactic acid bacteria is divided into two group, the homo-fermentative group which produces lactic acid as the sole products of fermentation of sugar, and hetero-fermentative which besides lactic acid also produces CO2, ethanol etc . The difference between the two is as a result of the absence of the enzyme aldolase in the heterofermenters .It contains approximately 125 specie including *L. casei, L. plantarumm, L. rhamnosus* and *L.acidophilus* It is predominantly mesophilic in nature and cannot usually resist high temperature.Lactic acid bacteria lack porhyrins and cytocromes, do not carry out electro transport phosphorylation and hence obtain energy by substrate level phosphorylation (okafor ,2007)

**2.5.3 FERMENTATION**

The term fermentation have come to have somewhat different meaning as it underlying cause have become better understood, the derivation of the word fermentation signifies a gentle bubbling condition .the term was first applied to the production of wine a thousand years ago .fermentation is the breakdown of sugar into alcohol.(Normam N. et al 2007)

Fermentation involve the use of microbial metabolic process in which carbohydrates and other nutrients are oxidized partially to a variety of breakdown products such as alcohol, acids amino acids and other metabolites. Fermentation is carried out for the modification or production of new or desired form of food( B. Sivansankar 2009)

The process of food fermentation involve mostly modification preservation by natural acidification mainly due to the formation of lactic acids.

Microorganisms used in food fermentation may be added as pure or mixed culture .in some cases the desired microorganism may be present in sufficient numbers in the original raw material.

Beneficial effects derived from the fermentation of foods include preservation; improved texture, color, flavor, and aroma; increased solubilization of proteins and digestibility, nutritional improvement, reduced cooking time, and removal of toxic substances (Whitaker, 1978). In most fermented high-protein foods, proteolysis is a major factor contributing to changes in texture and flavor. (Whitaker., 1978).Hydrolysis of lipids by microbial lipases provides substrates for fatty acid oxidation contributing to the characteristic flavor of various cheeses. Neutral carbonyl compounds produced by oxidation of the fatty acids in Cheddar cheese include formaldehyde, acetaldehyde, acetone, 2-butanone, 2-pentanone, 3-methyl butanol, 2-heptanone, 2- nonanone, 2-undecanone, 2-tridecanone, 2-pentadecanone, diacetyl, n-decanol, n-dodecanol and acetoin. The most significant flavor compounds found in cheddar cheese may be dimethyl sulfide, diacetyl and 3-hydroxybutanone (Whitaker.,1978).

**2.5.4 SENSORY ANALYSIS**

Sensory analysis of food is examined with the human sense to determine the organoleptic properties of a product, and the enjoyment of the products. Sensory science is the study of the reactions of the five senses, these are sight, hearing, smell, taste and touch. It helps to know the characteristics of physical matter. The „machines” are the human senses

The aim of sensory evaluation is to determine the food quality characteristics and the degree of compliance with the legal requirements and consumer habits.

The first and most important parameter of food is the sensory characteristics.It is complex property, and it is an opinion about the product itself, which cannot be replaced by any other method( Irfan Hashmi., 2007):

**2.5.5 PROXIMATE ANALYSIS**

 Proximate analysis is defined by H. Bennett in the Concise Chemical and Technical Dictionary as the "determination of a group of closely related components. It conventionally includes determinations of the amount of water, protein, fat (ether extract), ash and fiber, with nitrogen-free extract being estimated by subtracting the sum of these five percentages from 100. Many chemist use the word "crude" before protein, fat , and fiber in order to emphasize the group nature of their percentage. Since of these determinations are empirical, conditions of analysis must be precisely stated and followed. Results obtained in ash and moisture determinations are governed primarily by the temperature and the time of subjection to heat. Protein, fat and fiber figures do not represent single constituents. Any errors made in these five determinations are arithmetically cumulative in the figure obtained for nitrogen-free extract.

 **CHAPTER THREE**

 **3.0 MATERIAL AND METHODS**

Soybeans (glycin max)

Yogurmet (L.bulgaricus and L. acidophilus)

Dano milk(skimmed)

Sugar

Pineapple

Incubator

Water bath

Weighing balance

Bijou bottle

Sieve

Filter paper

 Autoclave

Refrigerator

**3.2 PROCEDURE FOR PRODUCTION YOGURT FROM SOYBEANS**

 **SOYMILK/ SOYBEANS PREPARATION**

The soybeans were purchased at Ogige Nsukka market. it was kept in a clean leather bag at room temperature.

The method as described by (.(Nig.J.Biotech. 1998) was employed for making soymilk.

The dry, mature, whole soybeans were steamed for 3 minutes to inactivate the enzyme,

lipoxygenase, and to loosen the hulls. The steamed soybeans were then washed and soaked overnight in distilled water at room temperature until at least 1 ml of H2O/g of dry weight was absorbed. Hulls were removed by hand under running water. The soaked, peeled soybeans were mixed with distilled water in a 1:5 ratio and blended in a Waring blender for 5 min. The resultant slurry was filtered through 3 layers of cheese-cloth. When filtering slowed, the remaining liquid was squeezed from cheese-cloth for 1 min. and the residue was discarded. The resultant soymilk was autoclaved at 121° C for 15 min., cooled, and stored at 50C.(Nig J.Biotech. 1998).table below shows soybean preparation

Sorting to remove the bad ones

Steaming for 3 minutes

Washing

Soaking over night

Removing of the hull

 Blending

Filtering

Autoclaving

Table 3.1 :Soymilk processing procedure

**3.3 PROCESSING OF PINEAPPLE**

Peeled to remove the back, they were washed and then blended using a food blender (Master chef blender, model no: MC– BL6731J). The pulp was then pasteurized for 85 0C for 3 minutes. It was then cooled. According into( Mbayi-Nwoha.,2003). Production and evaluation of flavored yogurt. Pineapple processing is shown in the table below

 Peeling

 Cut into pieces

 Blending

Pasteurization

 Cooling

***Table3.2:* Pineapple processing procedure**

***3.4 PROCESSING OF DANO MILK***

The sample of yogurt were produced according into international standard of yogurt as described by Guler and Mutlu (2005).Here 400g of skimmed powder dano milk was reconstituted with 7000 ml of water to obtain a creamy and uniform product pasteurization was carried out at 80 0C for 15 min as shown in figure 3 to destroy the undesirable microorganism and then allowed to Cool to 42-45 0C before inoculation with starter culture. The prepared pineapple sample were added. It was incubated at 450C for 24 hours. It is shown in the table below

 Skimmed milk

 Mixing (sugar and water)

 Homogenization

Pasteurization ( 800 C for 5 minutes)

 Cooling (42-450C)

 Addition of processed pineapple

 Inoculation of starter culture (yogurment)

 Fermentation

 Pineapple flavored yogurt

**Table3.4: Milk processing procedure**

 ***3.5 SOY-YOGURT PRODUCTION* OR FORMULATION OF PREMIXES**

The soy-yoghurt premixes were formulated to contain: (i) 100% soy milk without pineapple

(ii) 100% milk without pineapple, iii 100% soymilk with pineapple , 100% milk with pineapple

(Iv )50% dano milk and 50% soymilk, with pineapple (50: 50) ,(v) 50% soymilk and 50% dano without pineapple.

Yoghurt premixes formulated was divided into two portions and paturized. The mixture was

Subsequently placed in a water bath to cool down to 43°C the yoghurt mix was then dispatched into different ratios into the jars. The product was then cooled to temperature of 43 – 46 oC which is the ideal growth temperature of the starter culture. The pineapple pulp (already weighed) were added and the starter (yogurmet) inoculated. Fermentation was then carried out for 18 hours at 42 ± 2 0c after which yoghurt was set in retort bottles following stirring.

**3.5.1 SAMPLE ANALYSIS**

**SENSORY EVALUATION**

\The soy-yoghurt samples were kept at 6±2°C until evaluation. Panelist of 20 people were provided 10 female and 10 male. The sample was evaluated for acceptability, taste, color ,appearance and texture. They were instructed to rinse their mouth with water before the and after evaluation. All analysis were carried out in duplicate and data analyzed statistic package for social sciences (XPSS)

**DETERMNATION OF CRUDE PROTEIN**

The crude protein of the samples was determined by the micro Kjeldahl technique

(Pearson,1976). The method involve digestion of samples, distillation of digest and titration of

 distillate.

**PROXIMATE ANALYSIS**

The following proximate analysis were carried out on the formulated flavoured yoghurt and the nonflavoured yogurt.

**DETERMINATION OF MOISTURE**

The moisture content of the samples was determined using hot oven method of AOAC (2010).

The weight of the dry crucible was taken, then five millimeter (5 ml) of each of the sample was put into the crucible and weighed and placed into the Phoenix oven (Preiser model, New York,

USA) at a temperature of 70 - 80 0C for 2 hours and 100 – 105 oC until the weight is constant.

The samples were cooled in a desiccator and weighed.

The weight loss was obtained as the moisture content was calculated as:

; % Moisture content = W2-W3 X 100

 W1 1

Where; W1 = initial weight of empty crucible;

 W2 = weight of crucible + sample before drying;

W3 = final weight of crucible and sample after drying.

One millimeter (1ml) of the sample was put into a Kjeldahl flask.

Twenty five milliliter of H2SO4 with a catalyst which is a mixture of sodium sulphate

(NaSO4 ) and cupper sulphate (CuSO4)in the presence of selenium powder. The flask was then placed on an electrical coil heater in a fume chamber, this was gently boiled until a clear solution was gotten .The solution was then allowed to cool **distillation:** steam was passed through the Markham distillation apparatus for 10 minutes. About 10mls of boric acid indicator was added in a 50mls conical flask. The flask was placed under a condenser in a way that 10mls of the dilute digest was put in the distillation apparatus and rinsed down with 10mls of 40% sodium hydroxide (NaOH) before thr cup closed to prevent ammonium (NH2) from escaping.The steam was introduced for about 5 minutes until 20mls of the distillates was produced.

**Titratio**n: The distillates in the boric acid indicator was titrated with 0.01 N HCL to end point (a change in colour of brilliant green to pinkish colour)

%crude protein = T×14×01×0.01×6.25×100×10

 1×100

**DETERMINATION OF CRUDE FAT CONTENT**

250ml clean flask was dry in oven at 1050 C for about 45 minutes. The desiccator was cooled and the flask weighed Then, 2 milliliters of each of the samples were loosely wrapped with a filter paper and put into the thimble which was fitted to a clean round bottom flask, which has been cleaned, dried and weighed. The boiling flask was filled with 250ml n-hexane. The extraction thimble was plug with cotton wool . the soxhlet apparatus was set and reflux for 3 hours , the thimble was removed and the hexane was collected with care at the top container of the set upand drained into a container for reuse.

When the flask was almost free of the hexane,it was removed and dry at 1050C to a constant weight , it was transferred from the oven into desiccators , allowed to cool ,and then weighed percentage of the sample. % Fat Content = Weight of fat 100

Weight of sample × 1

**DETERMINATION OF ASH CONTENT**

A silica dish was heated to 6000 C, cooled in desiccators and weighed. Then 2ml of each sample was put into the silica dish place on the silica dish in a muffle furnace and heated for 3 hours.

Ash percentage is determined using: W3-W1 × 100

 W2 1

**DETERMINATION OF CARBOHYDRATE**

Carbohydrate content was determined by difference. It was done by subtracting the value of other food component (moisture, ash, fiber and protein) from 100 as described by Oyenuga (1968)Percentage carbohydrate = 100 - (% fat + % protein + %moisture + % ash +% crude fiber)

**CHAPTER FOUR**

 **4.0 RESULTS**

 **Table 4.1: AVARAGE SCORE OF SENSORY EVALUATION OF FORMULATION**

**Products** **food attributes**

SAMPLE COLOUR TASTE FLAVOUR TEXTURE OVERALL

100%M 6.44d±0.15 6.71c±0.04 5.76d±0.09 6.82d±0.11 5.95d±0.10

50%M&S 5.88b±0.11 3.12b±0.13 2.95c±0.11 5.94d±0.28 4.24d±0.16

50%M&S+P 43.94a±0.10 2.59a±0.24 2.29b±0.14 3.35b±0.09 2.76b±0.08

100%M+P 3.94e±0.10 2.71d±0.29 1.59d±0.06 2.14a±0.29 1.65c±0.14

100% S 2.95c±0.11 1.21e±0.28 1.3Oe±0.05 1.24c±0.06 1.59a±0.062

100%SP 2.59C±0.24 1.20f±0.20 1.10e±0.01 1.10c±0.04 1.24a±0.06

Number in roll with the same superscripts is significantly different at P<0.05 100%M = control sample (milk yogurt) 50% M&S = (50% dano milk and 50% soymilk)50% M&S +P =(50% dano milk and 50% soymilk with pineapple pulp) 100% M&P =( 100% dano milk and pineapple

pulp) 100%S = (100% soymilk)100%SP =(100%soymilk with pineapple pulp).

**4.1 PROXIMATE ANALYSIS RESULT**

**Table4.2: PROXIMATE COMPOSITION**

 **SAMPLES MOISTURE (%) ASH(%) FAT (%) PROTEIN(%) CHO(%)**

50%S+50%M 65.44 1.59 6.11 13.23 13.63

50%S+50%M+P 78.85 0.93 4.50 11.91 3.81

100%M 7.5.64 1.15 4.77 8.56 9.88

100%M+P 74.30 1.23 2.89 6.09 15.49

100%S+P 85.32 0.60 4.09 8.59 1.40

100%S 79.21 0.40 4.05 11.21 5.13

**CHAPTER FIVE**

**5.0 RESULT DISCUSSION**

**5.1 SENSORY EVALUTION**

**COLOUR**

The mean color rating for the samples ranged between 2.59 to 6.44 representing ‘dislike moderately to like very much’ on the 7-point hedonic scale. Sample 100%SP recorded the least mean rating while 100%M (control) recorded the highest mean score. Statistically there was no significant difference (p>0.05) between S and SP however they two were significantly different from the sample and the 50% M&P , 50%M&S+P. From the hedonic scale, it was evident that the panelist preferred the colour of the control sample and the 50%M&S but disliked the appearance of S and SP and this could be due to non-bright colour of soybean.

**TASTE**

 Taste refers to sensation perceived by the tongue which includes sweet, salty, sourness and bitterness (Goldsmith.,2008) This however could be influenced by the quality of the raw materials used in the processing of the yoghurt as well as the chemical reactions that might occur during the sequence of fermentation of the cultures used. With respect to the taste of the control and composite yoghurts, the mean ratings ranged from 1.20 to 6.71 indicating dislike moderately to like very much. The 100% M was the most preferred while SP was the least preferred by the panelists due to the high percentage composition of soy milk. Sample 100%M, 50M&S ,50%M&S+P, S milk and SP show significant difference in taste (p>0.05).

**TEXTURE**

Texture which could be termed as viscosity is likely to be influenced by the protein building blocks of the yoghurt gel network against the effect of fermentation time as well as the starter culture metabolism. Several researches reported that, products with greater viscosity and firmer texture have increased protein content. The mean scores for thickness ranged from 1.10 to 6.82 indicating dislike very much to like extremely. The most preferred sample was the control followed by composite yoghurt with 50% S&M. Significant differences existed in the texture for all samples of 50%M&S+P 100%M&P , 100%S and 100%SP(p<0.05).

**FLAVOR**

Flavor is often expressed as a combined sensation perceived through the chemical senses of taste and aroma as well as chemical irritation in the bucal cavity. Based on the hedonic scale, the mean values ranged between1.10 to 5.76 indicating dislike very much to like very much. Control sample was the most accepted product while sample 100%SP was the least accepted with respect to their flavor. Possibly the unpalatable beany flavour associated with soy bean imparted in the products and thus accounting for its relatively lower rating compared to the 100%M yoghurt. All samples were significantly different from each other.( Belleville., 2002)

**5.2 PROXIMATE COMPOSITION**

**MOISTURE**

From Table 2, the percentage moisture content of the samples ranged between 65.44and 85.32 with 100%SP recording the highest while 50%S&M recorded the least moisture content. Generally there was an increase in moisture content in the samples containing pineapple. This could be as a result of the water in the pineapple pulp( Opara et al.,2013)

**ASH**

The ash content represents the inorganic content in the samples. Ash content decreased correspondingly in the soy-yogurt and increased with the milk sample. This can be seen in the percentage ranges of the ash contents in 100%M(1.15) and sample 100%S(0.40) . The highest ash content is seen in sample 50%S&M (1.59) and the least in sample 100%S (0.40).

 **CRUDE FAT**

 The crude fat content ranged between 6.11 to 4.05 % with the sample 50%M&S recording the highest while sample 100%S recorded the least as shown in Table 2. The percentage of the fat content reduced with the introduction of pineapple to the sample decrease in fat content could be attributed to the pineapple being more of vitamin

**PROTEIN**

Parman stated that, properly defatted soybean flour will contain 50% or more of protein. As a fortification material, soybean stands a greater importance of increasing the protein value of food. From Table 2, the average percentage values of protein increased in the soy-yogurt samples than in the normal milk yogurt. 100%S (8.56) and 100%S (11.12). The protein content was equally decreased with introduction of pineapple. The protein percentage ranged from 13.23 to 6.09 respectively (Douglas.,2006)

**CARBOHYDRATE.**

The carbohydrate percentage ranged from 13.63 to 1 40 with the least of fat content being the sample 100%S+P. From the Table the fat content of the soy-yogurt is equally lesser than that of the normal yogurt

**CONCLUSION**

Using soymilk as a possible product in the manufacture of yoghurt so as to impact on the nutritive spectrum of consumers showed a relatively acceptable results. It was evident that, soymilk has the potential of giving an equal measure of nutritive and sensory value as would be obtained from an animal source having realized some incremental average values of the various organoleptic properties analyzed and in comparison to the pure (100%) dano milk yoghurt.

Though per this study it was established that no significant difference (P < 0.05) existed between the mean values of the samples with respect to the sensory evaluation of the composite yogurt, soy milk can be used to partially replace animal milk up to 50% in composite yoghurt without a possible organoleptic defect besides its nutritional and health benefits.

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